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Authors

Underwood, Mark A
Gaerlan, Stephanie
De Leoz, Maria Lorna A
et al.

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Human Milk Oligosaccharides in Premature Infants: Absorption, Excretion and Influence on the Intestinal Microbiota

Mark A. Underwood[1], **Stephanie Gaerlan**[2], **M. Lorna A. De Leoz**[2], **Lauren Dimapasoc**[2], **Karen M. Kalanetra**[3], **Danielle G. Lemay**[4], **J. Bruce German**[5], **David A. Mills**[3,5], and **Carlito B. Lebrilla**[2]

[1]Department of Pediatrics, University of California Davis, Sacramento, California, USA

[2]Department of Chemistry, University of California Davis, Davis, California, USA

[3]Department of Viticulture and Enology, University of California Davis, Davis California, USA

[4]Genome Center, University of California Davis, Davis California, USA

[5]Department of Food Science and Technology, University of California Davis, Davis, California, USA

Abstract

Background—Human milk oligosaccharides (HMOs) shape the intestinal microbiota in term infants. In premature infants, alterations in the intestinal microbiota (dysbiosis) are associated with risk of necrotizing enterocolitis and sepsis and the influence of HMOs on the microbiota is unclear.

Methods—Milk, urine, and stool specimens from 14 mother-premature infant dyads were investigated by mass spectrometry for HMO composition. The stools were analyzed by next-generation sequencing (NGS) to complement a previous analysis.

Results—Percentages of fucosylated and sialylated HMOs were highly variable between individuals but similar in urine, feces and milk within dyads. Differences in urine and fecal HMO composition suggest variability in absorption. Secretor status of the mother correlated with the urine and fecal content of specific HMO structures. Trends toward higher levels of *Proteobacteria* and lower levels of *Firmicutes*, were noted in premature infants of non-secretor mothers. Specific HMO structures in the milk, urine and feces were associated with alterations in fecal *Proteobacteria* and *Firmicutes*.

Conclusion—HMOs may influence the intestinal microbiota in premature infants. Specific HMOs, for example those associated with secretor mothers, may have a protective effect by

Corresponding author: Mark A. Underwood MD, Division of Neonatology, 2516 Stockton Blvd, Sacramento CA, 95817 USA; ; Email: munderwood@ucdavis.edu

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decreasing pathogens associated with sepsis and necrotizing enterocolitis while other HMOs may increase dysbiosis in this population.

Introduction

Human milk oligosaccharides (HMOs) are the third largest component of human milk. These complex carbohydrates are produced in a wide variety of structures that vary from woman to woman and within a given woman over time at great nutritional cost to the mother, and yet are not digestible by the infant([1]). This observation prompts the obvious evolutionary question: what is the benefit to the mother-baby dyad of these structures? HMOs appear to have multiple functions. First, they shape the composition of the infant intestinal microbiota through selective consumption by commensal bacteria([2, 3]). Bifidobacteria and *Bacteroidetes* become dominant intestinal bacteria in healthy breast-fed term infants due to their ability to digest and utilize HMOs via specific glycosidases, while most pathogenic *Enterobacteriaceae* lack these enzymes and are unable to utilize HMOs as a food source([4]). Second, HMOs have structural homology to many cell surface glycans and thus act as decoys by binding to luminal bacteria that are then unable to bind to the surface of the enterocyte([5]). Third, HMOs are absorbed into the vasculature([6]) and excreted into the urine([7]) potentially binding to and decreasing the capacity of invasive bacteria to cause sepsis and urinary tract infections([8]). Fourth, sialic acid containing HMOs may be important in neurodevelopment([9]).

Premature infants are at increased risk for necrotizing enterocolitis (NEC), sepsis, pneumonia, and neurodevelopmental delays due to immaturity and dysfunction of essentially every component of innate immunity. Premature infants who receive their mother's own milk have lower rates of NEC and sepsis than those that receive formula([10]). This is likely due to a variety of human milk components including immunoglobulins, lactoferrin, lysozyme and HMOs. Milk produced by women who deliver preterm differs from that of women delivering at term including the amounts and types of HMOs([11]). Women delivering preterm have much wider variation (both between women and for a given woman over time) in the percentage of HMOs that contain fucose or sialic acid than women who deliver at term([12]).

HMO structure is influenced in part by mutations in the maternal fucosyltransferase 2 (FUT2) gene. Individuals who are homozygous for mutations in the FUT2 gene (historically referred to as "non-secretors") are unable to produce α1,2 fucosylated glycans in their secretions including breast milk. Non-secretor individuals have lower levels of commensal bifidobacteria, are at decreased risk for infections with a variety of intestinal viruses, but have an increased risk for Crohn's disease([13]), celiac disease([14]) and NEC([15]). The influence of maternal secretor status on the infant microbiota has not been well characterized.

In this study, our objective was to determine the HMO composition of milk, urine, and feces from 14 premature infants receiving only human milk and their mothers and correlate these with the composition of the fecal microbiota. We hypothesized that ingested HMOs influence the intestinal microbiota.

Results

Relative abundances of the measured HMOs in milk, urine, and feces for all 14 dyads are summarized in Table 1 (the 4-digit labels correspond to the number of hexose, fucose, N-acetylhexosamine, and sialic acid residues, in that order. For example, the label 4120 (M=1220.47) means that there are 4 hexose, 1 fucose, and 2 N-acetylhexosamine residues in the structure). Representative mass spectra of milk from one of the mothers are presented in Supplemental Figures 1–3 (online) with a schematic diagram of HMO structure. The high degree of inter-individual variability is evident from the standard deviations in Table 1. Most dyads showed similarity in HMO groups across specimen type (ie milk composition was similar to fecal and urine composition). Figure 1 presents relative abundance of HMO groups for each dyad and Table 2 presents the combined data for all dyads. Total fucosylated, sialylated, fucosylated-sialylated, and non-fucosylated/non-sialylated HMOs were similar across specimen type (Supplemental Figure 4 (online)) and correlations between milk and fecal HMO groups were strong (Supplemental Figure 5 (online)). Individual HMOs however showed considerable heterogeneity. Structures relatively more abundant in feces than in urine included F-LSTc ($p<0.01$), LNFP II, 5130a, 5130c, and the group of 5 structures with mass 1731 ($p<0.05$) and TFLNH and 6'SL ($p=0.05–0.1$). We hypothesize that these HMOs are not significantly absorbed from the gut into the bloodstream. Several structures were not present in milk but found in either urine (3'Sle), feces (TFiLNO, 5330a), or both (A-hepta, 3'SLN). The origin of these structures is unclear; it is possible that these structures were present in earlier milk samples or that they were constructed or altered in the gut lumen by gut microbial enzymes. The only structure significantly more abundant in urine than feces was 3'Sle ($p<0.01$), though other structures showed a similar trend (3'SL, 3'SLN, LNFP I, LNFP V $p=0.1–0.2$). We hypothesize that these HMOS are absorbed from the gut into the bloodstream and filtered by the kidneys. The difference between 3'SL and 6'SL is striking as both structures have the same molecular weight with the difference in linkage apparently influencing absorption from the gut.

The secretor status of the mothers was predicted based on the percentages of 2'FL and LDFT ([16]). Of the 14 mothers enrolled 8 were secretors and 6 were non-secretors. Urine and fecal HMOs suggested a similar pattern in the infant to that of the mother's milk in all but infant number 13 (Table 3). The fecal microbiota was analyzed by terminal restriction fragment length polymorphism (TRFLP) in the original study ([17]). Based on the TRFLP data of all 14 specimens, infants of secretor mothers had lower *γ-Proteobacteria* ($p=0.046$) and higher *Lactobacillaceae* ($p=0.02$). For the current study, a more robust approach using next-generation sequencing (NGS) was performed on the 12 fecal samples for which sufficient sample remained and is summarized in Figure 2 and Supplemental Figure 6 (online). Note that the major HMO consuming orders of bacteria (*Bifidobacteriales* and *Bacteroidales*) are present in small numbers as has been previously demonstrated in premature infants. In this small sample, the differences by NGS in overall microbial composition between infants of secretor mothers and non-secretor mothers did not reach statistical significance, however the following trends were noted at the phylum level: increased *Firmicutes* ($p=0.08$) in the feces of infants of secretor mothers, and at the order

level decreased *Enterobacteriales* (phylum *Proteobacteria*, p=0.06) in the feces of infants of secretor mothers.

Correlations between HMOs in milk and fecal bacteria by NGS are presented at the phylum level in Figure 3 (HMO groups), Supplemental Figure 7 (online) (HMO groups, top panel) and Table 4 (individual HMOs). For many HMOs, the associations for the dominant phyla (*Firmicutes* and *Proteobacteria*) are in opposing directions. Figure 4 (top panel) summarizes correlations at the order level with the strongest correlations between HMOs in the milk and fecal *Enterobacteriales* (phylum *Proteobacteria*, positive correlation with HMOs containing neither fucose nor sialic acid and negative correlation with fucosylated HMOs) .

HMOs in the feces must traverse the length of the GI tract without being absorbed into the bloodstream or consumed by intestinal bacteria. In a pilot study of term breast-fed infants, as HMO consuming bacteria increased in the feces over time, fecal HMOs decreased in a structure specific manner (i.e. structures that are able to be consumed by the dominant bacteria decrease while those HMOs that are not digestible by the dominant bacteria persist) ([18]). In these preterm infants, correlations are noted between groups of HMOs in the feces (Supplemental Figure 7 (online), bottom panel) and individual HMO structures (Table 5) and fecal bacterial composition at the phylum level. At the order level, the persistence of fucosylated HMOs in the feces shows a positive correlation with *Pasteurellales*, *Pseudomonales*, and *Burkholderiales* and a negative correlation with *Enterobacteriales* (Figure 4, bottom panel). A detailed heat map summarizing Spearman's correlations for milk and fecal HMO groups and fecal bacteria at the genus level as determined by NGS in the current study is included as Supplemental Figure 8 (online). While the determination of absolute abundances of HMOs in the milk and feces is beyond the scope of this paper, previous studies have demonstrated that HMO content in feces is about 50% of that in milk([7]).

HMOs in the urine have been absorbed from the GI tract and filtered by the kidneys. Table 6 presents associations between urine HMOs and fecal bacteria as determined by NGS. Note that Tables 4–6 include only structures correlated with changes in fecal phylum with p values < 0.1, ie the structures not listed did not demonstrate significant correlations. While the determination of absolute abundances of HMOs in the urine is beyond the scope of this paper, previous studies have demonstrated that HMO content in urine is about 20% of that in milk([7]).

Discussion

Bifidobacteria and *Bacteroidetes* are the dominant fecal bacteria and the primary consumers of HMOs in healthy breast-fed term infants, but are generally rare in the feces of premature infants unless supplemented in the form of probiotics([19]). In these infants, bifidobacteria and *Bacteroidetes* were present in very limited amounts confirming previous observations of the marked differences in the fecal microbiota between term and preterm infants([20]). Bifidobacteria are associated with improved growth and vaccine responsiveness in term breast-fed infants in a developing country([21]). In premature infants, administration of probiotics containing bifidobacteria leads to a decreased risk of NEC, a common and

devastating inflammatory disease of neonates([22], [23]). In animal models and *in vitro*, bifidobacteria decrease inflammation and gut permeability([24], [25]). In this small group of premature infants (none of whom received probiotic organisms), the numbers of bifidobacteria were low (< 1% of all bacteria).

Enterobacteriaceae (phylum *Proteobacteria*) include pathogens that are associated with NEC and sepsis in premature infants such as *E. coli, Klebsiella, Enterobacter, Proteus,* and *Serratia*. These organisms are rare in the healthy adult gut, but common and often dominant in the intestine of premature infants. Colonization with *Klebsiella* in the first week of life([26]) and with *Enterobacteriaceae* in the second week of life([27]) have both been associated with increased risk of later development of NEC (which generally presents at 2–6 weeks of age). A bloom of these organisms has been demonstrated just prior to the onset of NEC in premature infants([28]). Some of these bacteria are capable of inducing an inflammatory response in the host and then utilizing the products of inflammation to outcompete other intestinal bacteria([29], [30]). The associations noted in these premature infants suggest that specific HMOs in human milk may decrease intestinal *Enterobacteriaceae* (e.g. fucosylated HMOs such as LDFT and LNFP V and structures that are both fucosylated and sialylated) while other HMOs in the milk are associated with increased intestinal *Enterobacteriaceae* (e.g. LNnH, 5031a and HMOs that contain neither fucose nor sialic acid). The mechanisms underlying these associations likely include both the host and interactions with other gut microbes as *Enterobacteriaceae* are unable to utilize HMOs as a food source([31]).

The impact of host secretor status on susceptibility to various infections is well documented and somewhat balanced in the adult population such that both secretors and non-secretors have differing advantages against specific pathogens. Recent demonstration of rapid fucosylation of glycans on the luminal surface of the enterocyte in response to stimulation of Toll-like receptors 2, 4, and 9 suggest this response (possible only in the secretor individual) may be important in protecting and nourishing gut commensals in the midst of infection([32]). In the premature infant, the inability of the non-secretor mother to provide key fucosylated HMOs appears to significantly influence the developing microbiota. The capacity of gut microbes to deconstruct specific linkages is a second promising mechanism underlying the observed associations. Recent studies have demonstrated marked differences between bacterial species in the capacity to consume specific HMOs([4]) and marked differences at the species level in production of specific glycosidases([33]).

Dysbiosis is often defined as alterations in the richness and diversity of the microbiota or more recently as delayed maturation of the microbiota of the infant([34]). While increased diversity of the microbiota may be beneficial in the adult, limited data suggest that prior to weaning a less complex microbiota that is dominated by bifidobacteria is associated with improved health (e.g. growth, thymus size, and vaccine response)([21]). Dysbiosis as a potentially alterable risk factor for NEC is based on several observations: increased risk of NEC with increased exposure to antibiotics([35]) and acid suppressing agents([36]) (both associated with dysbiosis), decreased risk of NEC with human milk([10]) and probiotics([22]) (both associated with less severe dysbiosis), and from direct observations([26]). While human milk decreases the risk of NEC, it appears to have less of an impact on the microbiota of the

premature infant than other factors such as antibiotic exposure and environmental factors([20], [37]).

The observed differences in absorption of specific HMO structures is consistent with previous observations([7]) and with the recent reports of HMOs in the plasma([6], [38]). Further exploration of this area is needed to determine any effects of HMO in the systemic circulation and/or urinary tract. In addition, if these differences are consistent it may be possible to develop ratios of urine:fecal HMOs as a marker of gut permeability for the infant (similar to the lactulose:mannitol test in current use for children and adults).

The number of mother-infant dyads included in this study is too small to make any firm conclusions, but is reasonable to generate hypotheses for testing in future larger studies. Perhaps the most compelling hypotheses generated by these data include the following: 1) that human milk rich in fucosylated HMOs is protective against dysbiosis while human milk rich in HMOs without fucose or sialic acid increases the risk of dysbiosis, 2) that milk from non-secretor mothers may not be as protective against dysbiosis and NEC as milk from secretor mothers (this hypothesis is consistent with observations of increased sepsis and NEC in non-secretor babies who likely have non-secretor mothers([15])) and 3) that premature infant fecal HMO composition is a biomarker, with feces high in HMOs containing sialic acid (e.g. 3'SL) and HMOs with neither fucose nor sialic acid associated with dysbiosis and feces high in fucosylated HMOs (particularly LDFT) associated with less severe dysbiosis. Given the observation that fucosylation is an immature and inconsistent process in the mammary gland of the mother delivering prematurely, these data also suggest the hypothesis that premature infants may benefit from supplementation with fucosylated HMOs (e.g. through pooled donor human milk). Future studies to confirm these observations and to correlate milk HMO composition and fecal HMO composition with short term outcomes (e.g. NEC and sepsis) and long-term outcomes (e.g. growth and neurodevelopmental outcomes) would be valuable.

Materials and Methods

We collected specimens of stool and urine from 14 premature infants enrolled in a clinical trial of prebiotic supplementation([17]) and milk from their mothers. The study was approved by the Institutional Review Board of the University of California Davis, informed consent was obtained prior to enrollment and the study was registered at clinicaltrials.gov (NCT00810160). Mean gestational age was 27 weeks at birth and 30 weeks at the time of enrollment. All infants were receiving enteral feedings of at least 100 ml/kg/d. Specimens described in this paper were obtained at baseline (prior to initiation of any supplement) from infants receiving exclusively their mother's own milk (no donor human milk). Specimens were frozen at −40° C upon collection and transported on dry ice for analysis. Extraction and analysis of HMOs in milk, urine and feces was performed as previously described([7]). The non-parametric Mann Whitney Rank Sum test was used to compare abundance of specific HMO structures in urine and stool and linear regression was used to correlate HMO abundance and fecal microbial abundance. As this study is predominantly a hypothesis generating exercise we reported all p values <0.1.

Bacterial DNA was extracted from the stool specimens as described in the clinical trial([17]), however in addition to the analyses by polymerase chain reaction and terminal restriction fragment length polymorphism (TRFLP) previously presented, the samples were analyzed by next generation sequencing for this paper. For these analyses, DNA library construction was carried out as previously described ([39]) and submitted to the UC Davis Genome Center DNA Technologies Core for sequencing on an Illumina MiSeq instrument (Illumina, San Diego CA). QIIME software package (University of Colorado. Boulder CO, version1.7.0) was used for quality filtering and demultiplexing the resulting sequencing data([40]). OTUs were assigned using UCLUST (drive5.com, Tiburon, CA) based on 97% pairwise identity([41]) and taxonomic classification was based on the Ribosomal Database Project classifier (Michigan State University, East Lansing, MI) against a representative subset of the Greengenes 16S rRNA database (Second Genome, South San Francisco, CA, gg_otus_12_10 release)([42], [43]). Taxonomic relative abundance data from the QIIME data analysis was used to calculate correlations between HMO consumption and fecal bacteria abundance.

To visualize correlations between HMO consumption and fecal bacteria, custom R scripts were developed. HMO or bacterial populations with a maximum abundance less than 0.02 were removed from further analysis. A Spearman’s correlation matrix was computed using the remaining HMO and bacteria features. The procedure was repeated at the phylum, order, and genus levels. We chose to use the Spearman's correlation because it is a nonparametric measure that is robust to outliers and does not require that the data be normally distributed.

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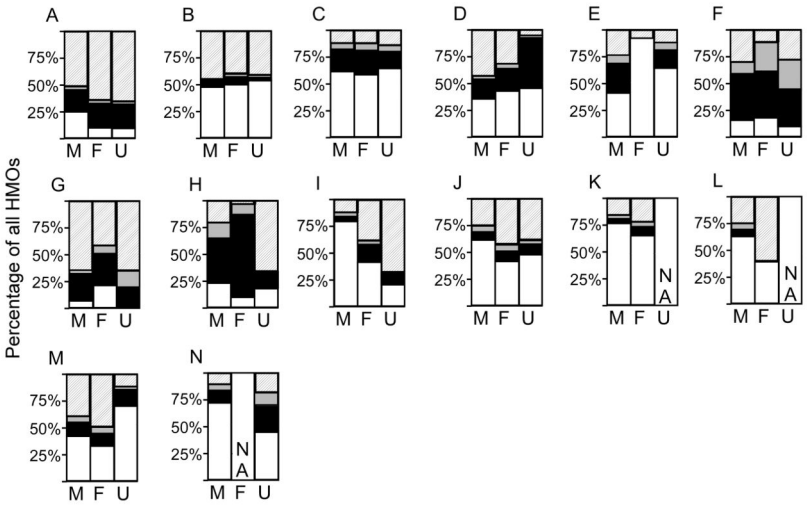

Figure 1.

Relative abundance of groups of HMOs in the milk, urine, and feces for each of the 14 mother-infant dyads. White: fucosylated HMOs, black: sialylated HMOs, grey: fucosylated and sialylated HMOs, hatched: HMOs with neither fucose nor sialic acid. Panels A=patient 4, B=patient 6, C=patient 9, D=patient 10, E=patient 13, F=patient 14, G=patient 16, H=patient 18, I=patient 20, J=patient 21, K=patient 22, L=patient 26, M=patient 27, N=patient 30. M=milk, F=feces, U=urine, NA=specimen not available.

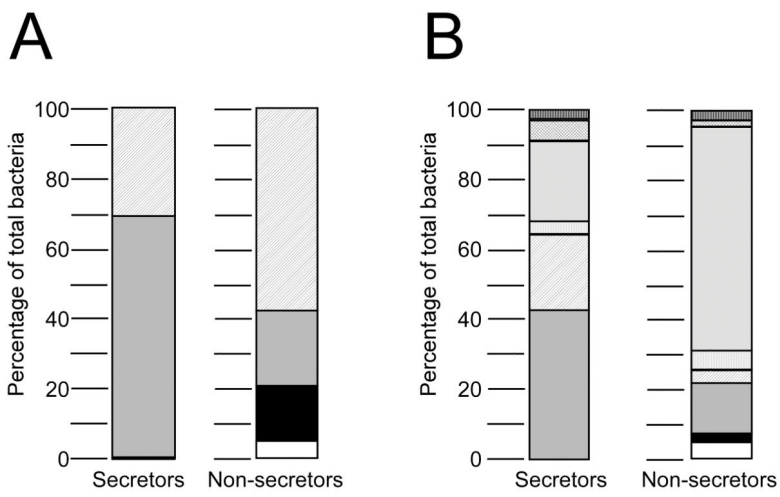

Figure 2.

Composition of the fecal microbiota determined by Next Generation Sequencing in twelve of the infants (6 with secretor mothers and 6 with non-secretor mothers). A. Phylum level, White: *Actinobacteria*, black: *Bacteroidetes*, grey: *Firmicutes*, hatched: *Proteobacteria*. B Order level. White: *Bifidobacteriales*, black: *Bacteroidales*, grey: *Bacillales*, hatched: *Lactobacillales*, dots: *Clostridiales*, light grey: *Enterobacteriales*, double hatched: *Pseudomonadales*, vertical stripes: others.

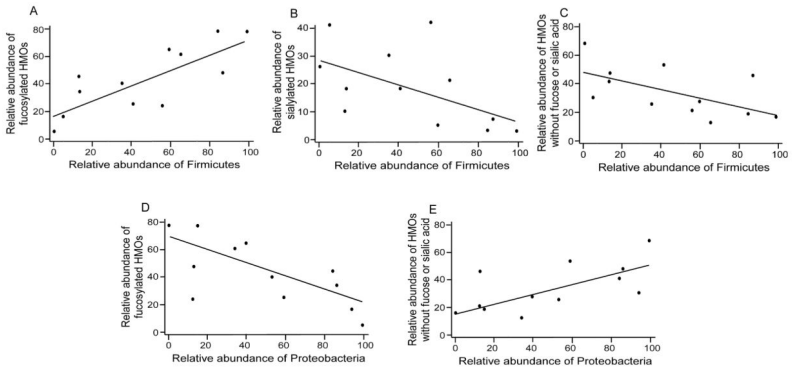

Figure 3.

Linear regression associations between groups of milk HMOs and fecal microbes by phylum (n=12). A. R^2=0.63, p=0.002. B. R^2=0.31, p=0.06. C. R^2=0.36, p=0.04. D. R^2=0.48, p=0.01. E. R^2=0.42, p=0.02.

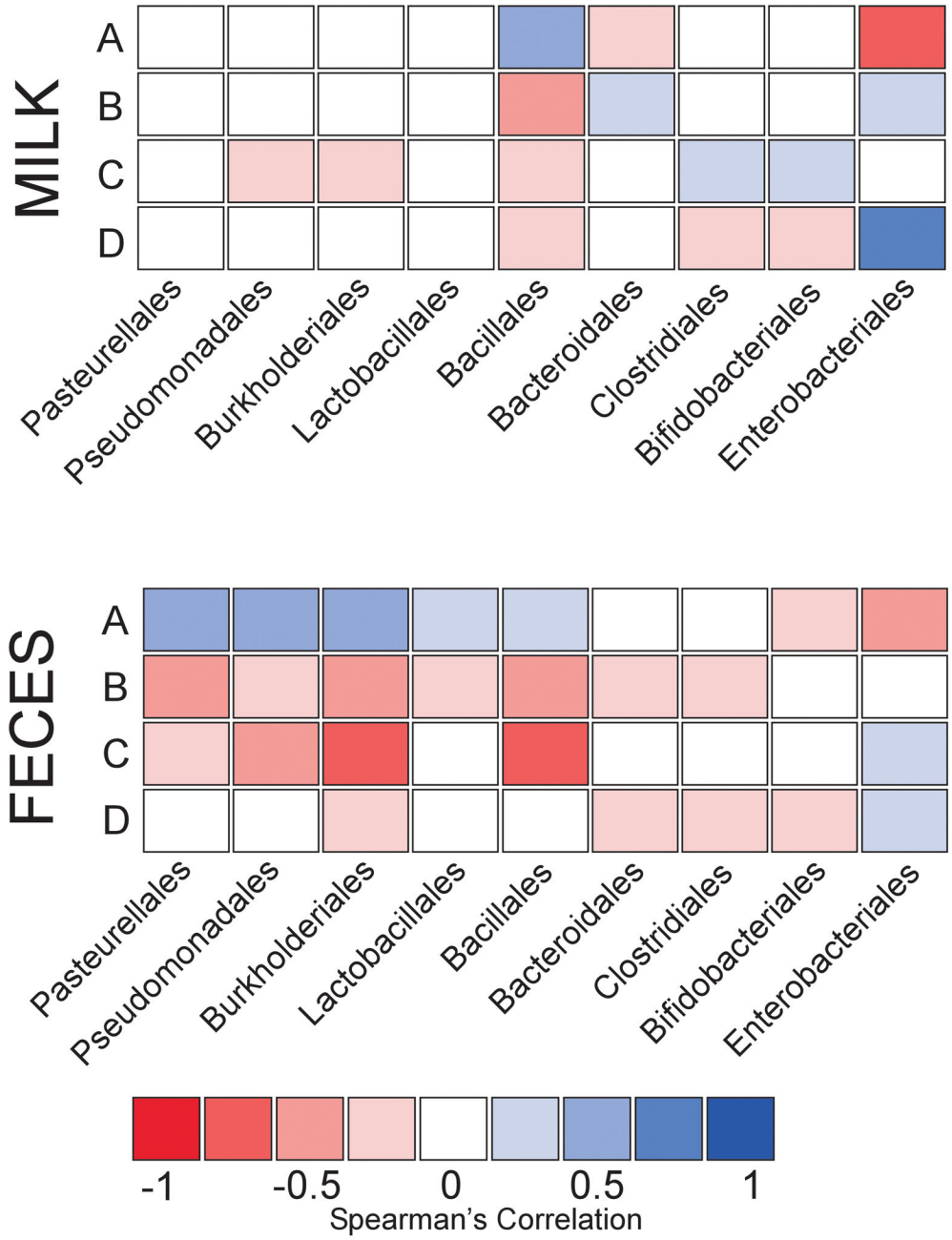

Figure 4.

Heat map demonstrating correlations between milk HMO groups and fecal bacterial orders (top) and between fecal HMO groups and fecal bacterial orders (bottom). Colors represent strength of correlations with deep blue representing Spearman's rho=1 and deep red representing Spearman's rho=−1.

Table 1

Mean percentage of HMO structures in the milk, urine, and feces.

Structure	Mass	Milk (N=14), Mean% (SD)	Urine (N=12), Mean% (SD)	Feces (N=13), Mean% (SD)
3'FL	490.1893	0.277 (0.647)	0.761 (1.65)	0.0546 (0.0988)
2'FL	490.1908	22.0 (22.3)	8.67 (11.4)	9.22 (12.9)
LDFT	636.2476	2.63 (3.73)	5.10 (6.64)	3.06 (3.40)
LNT/LNnT	709.2654/709.2655	22.6 (17.5)	29.6 (25.0)	25.0 (17.1)
LNFP II	855.3199	1.67 (2.46)	0.779 (1.91)	4.12 (5.15)
LNFP III	855.3225	0.614 (1.22)	0.201 (0.406)	1.17 (1.99)
LNFP I	855.3233	0.764 (1.64)	4.78 (9.15)	0.767 (1.87)
LNFP V	855.3223	7.24 (9.87)	12.3 (13.9)	4.63 (9.03)
LNDFH I/LNDFH II	1001.378/1001.3813	2.47 (5.90)	1.22 (2.09)	7.04 (11.7)
LNH	1074.4017	3.73 (2.77)	1.90 (3.16)	2.03 (1.55)
LNnH	1074.3946	2.20 (1.82)	1.75 (2.44)	1.48 (1.48)
pLNH	1074.3957	1.47 (1.79)	0.445 (0.497)	1.48 (1.99)
A-hepta	1204.468	0 (0)	0.0193 (0.0670)	0.116 (0.410)
6 structures[a]	1220.455-1220.474	4.45 (4.12)	6.45 (7.63)	6.13 (3.41)
4 structures[b]	1366.510-1366.522	2.42 (3.09)	3.30 (4.80)	3.53 (2.34)
TFLNH	1512.576	0.210 (0.233)	0.138 (0.265)	0.424 (0.517)
5130a	1585.583	0.566 (0.817)	0.0113 (0.0392)	0.611 (0.832)
5130b	1585.585	0.112 (0.173)	0.326 (0.417)	0.478 (0.382)
5130c	1585.585	0.150 (0.262)	0.0742 (0.156)	0.289 (0.326)
F-LNO	1585.588	0.550 (0.585)	0.183 (0.431)	0.338 (0.356)
6 structures[c]	1731.642-1731.649	0.632 (0.757)	0.274 (0.584)	1.29 (1.00)
TFiLNO, 5330a	1877.699-1877.702	0 (0)	0 (0)	0.104 (0.260)
6140a	1950.719	0.224 (0.369)	0 (0)	0.178 (0.328)
6'SL	635.229	1.81 (2.35)	0.803 (2.78)	4.62 (6.43)
3'SL	635.227	1.01 (0.878)	4.62 (8.16)	0.865 (1.24)
6'SLN	676.254	0.0454 (0.0916)	2.31 (3.52)	0.823 (2.33)
3'SLN	676.252	0 (0)	1.68 (4.16)	0.00230 (0.00827)
3'Sle	822.314	0 (0)	0.085 (0.116)	0 (0)
LSTa/b/c	1000.362-1000.365	11.0 (10.8)	6.35 (5.39)	11.8 (12.2)
F-LSTc	1146.419	1.35 (2.08)	0.0654 (0.150)	1.36 (2.24)
DSLNT	1291.446	0.239 (0.457)	0.412 (0.543)	0.242 (0.618)
S-LNH	1365.495	0.490 (0.725)	0.484 (0.449)	0.296 (0.368)
4021a	1365.494	0.715 (0.980)	0.570 (1.97)	0.768 (0.979)
S-LNnH II	1365.494	1.97 (2.16)	0.962 (1.53)	1.08 (1.22)
4021b	1365.491	0.221 (0.287)	0.151 (0.284)	0.166 (0.213)
7 structures[d]	1511.547-1511.554	3.13 (2.27)	2.68 (2.73)	3.38 (2.71)

Structure	Mass	Milk (N=14), Mean% (SD)	Urine (N=12), Mean% (SD)	Feces (N=13), Mean% (SD)
DFS-LNH	1657.612	0.234 (0.214)	0.183 (0.279)	0.190 (0.295)
5031a	1730.622	0.203 (0.251)	0.113 (0.188)	0.230 (0.268)
FS-LNO/5131a	1876.682	0.108 (0.142)	0.0456 (0.745)	0.259 (0.368)
6041a	2095.76	0.0455 (0.0703)	0 (0)	0.0996 (0.179)

The structures underlined and highlighted in bold are found in milk and are more than 5 fold more abundant in urine than feces. The structures underlined and in italics are found in milk and are more than 5 fold more abundant in feces than urine.

The following structures were detectable in at least one specimen of milk, urine and feces with a mean of less than 0.1%: 4320a, Tetra-iso-LNO

The following structures were not detected in any milk or urine samples, but were detected in at least one fecal sample at a mean of less than 0.1%: 6340a, 6340b, 6340c, 6440a, 5231a, 5231b, 5331a.

The following structure was not detected in any urine samples, but was detected in at least one milk and and one fecal sample at a mean of less than 0.1%: 6240a

[a] MFpLNH IV, 4120a, MFLNH III, MFLNH I, IFLNH III, and IFLNH I

[b] DFpLNH II, DFLNHb, DFLNHa, DFLNHc

[c] DFLNO I, DFLNnO II, 5230a, DFLNnO I, DFLNO II, 5230b

[d] 4121a, 4121b, FS-LNH III, FS-LNH, FS-LNH I, FS-LNnH I, FS-LNH II

Table 2

Mean percentages of groups of HMOs in milk, urine and feces.

Structure	Milk (N=14), Mean% (SD)	Urine (N=12), Mean% (SD)	Feces (N=13), Mean% (SD)
Fucose + Sialic acid	4.91 (3.81)	3.26 (3.32)	5.60 (5.52)
Fucose	46.9 (24.0)	40.3 (21.4)	41.5 (22.9)
Sialic acid	17.0 (13.6)	20.1 (13.0)	19.8 (20.2)
No Fucose or Sialic acid	31.2 (16.9)	36.3 (24.5)	33.1 (20.1)

Table 3

Predicted maternal secretor status and associated infant urine and fecal HMOs from each mother-baby dyad. Sec=secretor, Non-Sec=non-secretor, NA=specimen not available

Infant	Milk 2'FL	Milk LDFT	Predicted Maternal Secretor Status	Urine 2'FL	Urine LDFT	Feces 2'FL	Feces LDFT
4	0%	0%	Non-Sec	0%	0%	0%	0%
6	6%	3%	Sec	0%	5%	0%	7%
9	50%	4%	Sec	27%	16%	27%	5%
10	24%	0%	Sec	12%	1%	18%	1%
13	2%	0%	Non-Sec	10%	19%	7%	2%
14	1%	0%	Non-Sec	0%	0%	0%	0%
16	1%	0%	Non-Sec	0%	0%	0%	0%
18	2%	0%	Non-Sec	0%	0%	0%	0%
20	46%	11%	Sec	34%	9%	13%	8%
21	40%	0%	Sec	14%	8%	10%	10%
22	47%	4%	Sec	NA	NA	42%	4%
26	33%	4%	Sec	NA	NA	3%	4%
27	0%	0%	Non-Sec	0%	0%	0%	0%
30	56%	10%	Sec	7%	2%	NA	NA

Table 4

Associations between milk HMOs and fecal bacterial phyla.

Milk HMO	Fecal Bacteria	Correlation	R-squared	P value
2’FL	Proteobacteria	negative	0.27	0.09
LDFT	Proteobacteria	negative	0.42	0.02
LNFP V	Proteobacteria	negative	0.37	0.04
LNnH	Proteobacteria	positive	0.37	0.04
5031a	Proteobacteria	positive	0.35	0.04
2’FL	Firmicutes	positive	0.40	0.03
LDFT	Firmicutes	positive	0.56	0.005
LNFP V	Firmicutes	positive	0.51	0.009
LNH	Firmicutes	negative	0.34	0.05
LNnH	Firmicutes	negative	0.34	0.05
4120 (six structures)	Firmicutes	negative	0.25	0.09
TFLNH	Firmicutes	positive	0.29	0.07
3’SL	Firmicutes	negative	0.28	0.08
5031a	Firmicutes	negative	0.32	0.05

Table 5

Associations between fecal HMOs and fecal bacterial phyla.

Fecal HMO	Fecal bacteria	Correlation	R-squared	P value
LDFT	Proteobacteria	negative	0.40	0.03
LNH	Proteobacteria	positive	0.35	0.04
4120 (six structures)	Proteobacteria	positive	0.41	0.03
5130a	Proteobacteria	positive	0.63	0.002
5230 (five structures)	Proteobacteria	positive	0.45	0.02
6140a	Proteobacteria	positive	0.25	0.098
3'SL	Proteobacteria	positive	0.29	0.07
5031a	Proteobacteria	positive	0.27	0.08
6041a	Proteobacteria	positive	0.36	0.04
LDFT	Firmicutes	positive	0.55	0.006
4120 (six structures)	Firmicutes	negative	0.26	0.09
5130a	Firmicutes	negative	0.58	0.004
5230 (five structures)	Firmicutes	negative	0.36	0.04
6140a	Firmicutes	negative	0.25	0.099
6041a	Firmicutes	negative	0.31	0.06

Table 6

Associations between urine HMOs and fecal bacterial phyla.

Urine HMO	Fecal bacteria	Correlation	R-squared	P value
Sialylated HMOs	Proteobacteria	positive	0.35	0.05
p-LNH	Proteobacteria	positive	0.34	0.06
5130b	Proteobacteria	positive	0.30	0.08
3’Sle	Proteobacteria	positive	0.29	0.09
Sialylated HMOs	Firmicutes	negative	0.39	0.04
2’FL	Firmicutes	positive	0.33	0.07
p-LNH	Firmicutes	negative	0.35	0.05
5130b	Firmicutes	negative	0.31	0.08
3’Sle	Firmicutes	negative	0.28	0.09